NYU-EPA PM CENTER:

HEALTH RISKS OF PM COMPONENTS

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PROGRESS REPORT

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The NYU-EPA PM Center operates by funding individual research projects broadly aimed at determining the role which specific physicochemical properties of ambient PM may play in the production or exacerbation of adverse health effects. These projects are in the areas of exposure assessment, epidemiology, clinical (human controlled exposure) research and animal toxicology.

EXPOSURE ASSESSMENT STUDIES

Exposure Characterization Errors

PI: K. Ito (NYU)

Objective

The objectives of this project are threefold: (1) To quantitatively characterize spatio-temporal error of PM components and gaseous co-pollutants measured at routine regulatory-based air monitors as a function of site characteristics using the entire US air monitoring network; (2) To establish the relationship between the estimated error at a given monitoring site and the effect size / significance in mortality and morbidity models; and (3) To evaluate the relative contribution of the error due to site-to-site and person-to-site variability. This project will test the prevailing hypothesis that the PM and gaseous co-pollutants data from a single air monitoring station can adequately reflect the population exposure for the entire city, and that resulting risk estimates and their significance are not biased.

Progress

In the first year of this project, we processed much of the nationwide air pollution and mortality records. In the first half of the 2nd year, we extended our study period from 10 years to 13 years because we could obtain mortality records for three additional years (1995-1997) than originally planned (1985-1994). The daily PM_{10} data, hourly O_3, SO_2, and CO data for all the sites in the contiguous U.S. states for the 13-year study period have been retrieved and processed. For the gaseous pollutants, daily 24-hr averages were computed for all the sites. There were 2,368 sites for PM_{10}, 1,544 sites for O_3, 1,473 sites for SO_2, and 904 sites for CO for the entire study period. Although PM_{10} had the largest number of sites, a smaller number of sites had a large number of available days because of the every-6th-day sampling schedule used at most sites. However, 25% of the PM_{10} sites (590 sites) had at least 565 days available (enough daily data to conduct time-series study with a reasonable statistical power), and 10% (236 sites) had over 900 days of observations available. As the gaseous pollutants (i.e., O_3, SO_2, and CO) were collected on a daily basis, larger numbers of daily observations were available. For example, 25 % of O_3 (386 sites), SO_2 (368 sites), and CO (226 sites) had at least 3,100 days of observations. Data analysis has started using PM_{10} and gaseous pollutants in some of the larger cities, e.g., Detriot, Chicago. Preliminary results suggest that monitors with low correlation tend to have smaller mortality relative risks in some of these cities.

We decided to further extend the study period, because many ambient monitors started collecting $PM_{2.5}$ data in 1999. Nationwide $PM_{2.5}$ data for the period 1999-2000 have been retrieved and processed. While the collection period for much of the $PM_{2.5}$ data is shorter than that for PM_{10}, many of these $PM_{2.5}$ monitors collected data every-3rd-day (as compared to every-6th-day for most of the PM_{10} data) or even every day, providing the sample size sufficient to conduct data analyses for the first objective above, and even for the second objective above for the cities with large population size. Thus, taking advantage of the newly available $PM_{2.5}$ data, while delaying the original schedule set for the more limited PM_{10} and $PM_{2.5}$ data, would greatly enhance the overall research goal, adding a more powerful comparison of the PM_{10} and $PM_{2.5}$ data.

Plans for Next Year

Data analyses for the extended study period (1985-2000) for air pollution data will be continued. As originally planned, characterization of air pollution data (e.g., monitor-to-monitor temporal correlation) will be conducted for the nationwide data. However, more detailed characterization and comparison of the PM_{10} and $PM_{2.5}$ data will be conducted in areas where extensive data for both of these PM indices exist from multiple monitors. Mortality and morbidity (the elderly hospital admission) data analyses will be conducted in these areas up to 1999 (2000 for the elderly hospital admission data). Finally, since an ongoing EPA-funded NYU PM personal exposure study (Lippmann, P.I.) has finished collecting data in NYC, we will be able to compare the magnitude of the error associated with person-to-monitor differences with that associated with monitor-to-monitor variability.

X-ray CT-based Assessment of Variations in Human Airway Geometry: Implications for Evaluation of Particle Deposition and Dose to Different Populations

PI: B.S. Cohen (NYU); E.A. Hoffman (University of Iowa)

Objective

Few data are available regarding the regional deposition of particulate matter in the lungs of people with respiratory diseases and the normal elderly, subpopulations which may be at special risk of environmentally-related lung disease. This project seeks to investigate the potential for retrieval of morphometric data from three dimensional images of conducting airways obtained by x-ray computerized tomography (CT), and to explore the potential for the use of stereolithography (STL) to produce hollow airway casts for experimental verification of particle deposition models. It also will test and validate theoretical and empirical models used to predict detailed particle deposition in living individuals and, ultimately, will allow application of the derived, validated dosimetry models to airways representative of various potentially susceptible subgroups.

Progress

Rapid prototype processing is necessary to create a physical (solid) model of the segmented airway lumens. Shape-based interpolation was used to create isotropic voxels and to smooth the surface of an airway model. A volumetric rendering of the resultant segmented luminal space of the airway tree phantom was generated utilizing a marching cubes algorithm. Triangular patches divide the cube between regions within the isosurface and regions outside of airway tree. By connecting the patches from all cubes on the isosurface boundary, we produced a surface representation of airway tree. These triangular patches were converted to an STL or stereolithography file format required by the rapid prototyping device.

The stereolithography unit uses a computer controlled arm connected to a plastic extrusion device to build volumetric structures layer-by-layer. Two heads are present on the machine, one to lay down the plastic compound for the structure of interest and a second head to lay down needed support material for the structure as it is being built and which can later be separated from the structure.

Thin multi-slice helical CT scanning allows the acquisition of high resolution volumetric image data sets of the lung in a breath-hold or at multiple phases within a respiratory cycle. From these scans, hollow airway casts that include 5 or 6 bronchial generations were created and replicated for potential use in studies of inhaled particle deposition.

Plans for Next Year

During the next year, we will begin to utilize the accomplishments to date, which provided the tools for utilization and retrieval of morphometric data from three dimensional

images of human lung conducting airways obtained by volumetric x-ray. The ultimate goal will be to quantify the impact of airway variability on PM deposition and dose. We will begin to compare inhaled particle deposition pattern and efficiency *in vivo* in sheep with deposition measured in a hollow airway cast prepared from a three dimensional image of sheep lungs so as to validate the physical model system. We will also explore deposition for a variety of breathing patterns, and when particles are inhaled at different points in the respiratory cycle. We will also begin to examine bolus deposition *in vivo* in order to validate the hypothesis that regional deposition can be predicted via a mathematical algorithm based on a progressive series of varying-depth bolus deposition measurements.

EPIDEMIOLOGY STUDY

Asthma Susceptibility to PM

PI: G. Thurston (NYU); J. Reibman (NYU)

Objective

The objectives of this project are to investigate which PM component(s) and PM mechanisms affect asthmatics most strongly, and to prospectively follow a cohort of non-smoker asthmatics and evaluate PM effects on their health status. The ultimate goals are to establish technical and operational feasibility for a combined epidemiological/clinical research study; demonstrate associations between specific PM components and commonly occurring asthma exacerbations attributable to air pollution; and develop hypotheses regarding the mechanisms of the PM-health effects association that can be tested via toxicological studies by other researchers in the NYU-EPA PM Research Center (e.g., via controlled exposure studies). Moreover, the results of this study may be used as preliminary results for the funding of a follow-up study in this already characterized population.

Progress

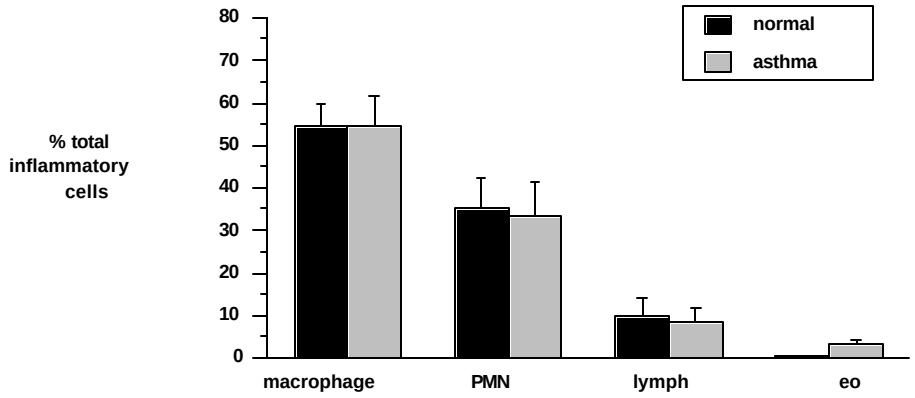
In the winter of 1999-2000, recruitment continued of patients for our cohort of adult non-smoking asthmatic subjects willing to be followed by prospective monitoring, on days following low vs. high PM2.5 concentrations. Because of difficulties in the first summer (of 1999) in inducing sputum in asthma patients, we felt we needed to improve our induced sputum technique. Approval was obtained to induce sputum from normal volunteers, and 10 subjects were recruited and duplicate procedures performed on these subjects.

Subjects with asthma were recruited from the previous summer cohort, clinics and local advertisements. Participants were asked to be "on call" for 1 day notice to come for 4 visits, 2 "High" and 2 "Low" visits. These correspond to 2 day lag visits from the defined day. Subjects then underwent pulmonary function testing (PFT), blood draw, pre-medication with bronchodilator, followed by sputum induction. "High" and "Low" PM days were defined

based on analysis of previous data: "High" = $PM_{10} \geq 40\mu g/m^3$, while "Low" = $PM_{10} \leq 20\ \mu g/m^3$.

Sputum induction was performed by use of increasing concentrations of hypertonic saline that were inhaled (3%, 4%, 5%) for 7 minutes, via an ultrasonic nebulizer. Subjects underwent spirometry for measurement of FEV_1 at the start of the procedure, and after each period of inhalation. If the FEV_1 dropped 20%, the procedure was terminated. After each saline inhalation, subjects coughed into a sterile container. Sputum plugs were separated from saliva and examined within 2 hours. After weighing, sputum plugs were dissolved in dithiothreitol (0.1%) and phosphate buffered saline. The suspension was then filtered and a total nonsquamous cell count performed. Cell viability was determined by trypan blue exclusion. Cytospins were prepared, stained with Wright's stain, and a differential cell count of nonsquamous cells types performed. Metachromatic cells were detected in preparations stained with toluidine blue. Cell pellets were also prepared for RNA analysis.

To date, sputum samples have been successfully collected on both normal subjects (n=10) and from subjects with asthma (n=11). In addition, some 44 blood serum samples were collected. This is not yet sufficient for the originally envisioned high vs. low PM day comparisons. However, these samples can provide a basis for evaluating which biomarkers can be successfully used to assess PM-induced effects in the study population. For example, as shown below, preliminary findings from several of these samples have already demonstrated the ability to detect and measure inflammatory cells in sputum samples, as well as the presence of elevated levels of eosinophils. In addition, sputum samples were analyzed for the presence of dendritic cells (CD1a+), and the quality of mRNA was tested in sputum cell pellets.



Overall, progress was made toward our study goals, but practical problems arose limiting our success in achieving the originally proposed results within the anticipated timeframe. The number of subjects that reliably participated was too limited, and very few days in the rainy summer of 2000 met our "high" PM pollution day criteria. Only 2 days in the summer of 2000 met the "high" pollution day criteria, as opposed to an expected 18 days. Furthermore, only 50% of our previous subjects agreed to return for the study. Forty subjects were screened by PFT and clinical parameters. Twenty of these subjects failed screening on

PFT criteria even after modification of exclusion criteria; 13 patients agreed to participate in the screening. These factors conspired to significantly reduce the number of sample-days that could be collected.

Plans for Next Year

The limitations in our ability to collect and analyze samples forced us to re-examine our results to-date and adjust our approach in order to better work towards achieving our planned goals. We plan to analyze the paired sputum and serum samples that we have collected from both normals and subjects with asthma to date, in order to assess those specific indicators that can be used successfully as asthma biomarkers. In particular, we propose to follow up on some recent animal studies by Dr. J. Zelikoff (NYU PM Center), which demonstrate that inhalation of concentrated ambient PM alters circulating immune blood cell profiles in a manner indicative of a stress response. Thus, we plan to analyze this limited number of sputum and serum samples for assessing acute inflammatory cytokine markers, including IL-1,6,8,10 and tumor necrosis factor. In addition, metallo-proteinases derived from inflammatory and structural cells that have been reported in serum (e.g., MMP-9 and TIMP) will also be evaluated in these samples.

Based upon our findings from the already collected samples, new samples will be collected weekly on a limited number of asthma subjects during the summer of 2001 (e.g., 10 weekly, at 2 per day during Monday-Friday). This scheduled design is expected to avoid past problems experienced in trying to bring in subjects on short notice. Those cytokines that we will, by that time, have shown can be meaningfully evaluated in these samples, as well as changes in immune cellular responses (including lymphoproliferation), will be determined in both serum and sputum media. Moreover, if effects upon neutrophil emigration in human subjects are found to be similar to those previously observed by Dr Zelikoff in her separate animal model studies, additional endpoints will be examined in the sera, potentially including soluble TNF receptor and adhesion molecules. This new approach, therefore, will utilize results from the data that we have collected to date, as well as address past data collection problems in the original study design.

CLINICAL EXPOSURE STUDY

Health Effects of Ambient Air PM in Controlled Human Exposures

PI: T. Gordon (NYU); J. Reibman (NYU)

Objective

The hypothesis being evaluated in this project is that concentrated ambient PM will produce acute adverse respiratory and cardiovascular health outcomes in volunteers under controlled exposure conditions.

Progress

Little progress has been made in the actual exposure of human subjects to concentrated ambient PM, due largely to technical problems in the development of a new higher volume centrifugal concentrator. Thus, while the original study endpoints spanned a spectrum of physiological, cellular, and biochemical responses useful for determining the cardiopulmonary effects of PM exposure, we have recently scaled back on these endpoints.

Due to the fact that the human exposure project was not moving at its scheduled rate, we concentrated (in collaboration with Dr. L. Chen) on efforts examining the *in vitro* response of human bronchial epithelial cells to size-fractionated ambient PM. Because of the significant association between ambient PM and exacerbation of allergic asthma, we examined the potential for airway epithelial cells (primary culture) to modulate the immune system. Size-fractionated ambient PM was collected with a MOUDI impactor for 2 weeks intervals throughout the year and used to treat human bronchial epithelial cells obtained from normal human volunteers. The fraction of particles less than 0.18 μm (i.e., primarily the ultrafine fraction) produced a dose-dependent increase in GM-CSF released from the epithelial cells. GM-CSF is a cytokine that can elicit inflammation in the airways via an effect on eosinophils, and can also modulate immune responses via effects on dendritic cells. There was no change in secreted GM-CSF in cells treated with larger sized ambient particles or equivalent doses of carbon or Mount St. Helen dust particles, thus suggesting that the human epithelial cell response was not due to a general particle effect. Moreover, treatment of epithelial cells with endotoxin had no effect on GM-CSF. Further experiments with inhibitors demonstrated that MAPK pathways are involved in the ambient particle effects on GM-CSF secretion by epithelial cells.

Plans for Next Year

Normal subjects (ages 18 to 50) will be exposed for 2 hours by face mask to filtered air or 150 $\mu g/m^3$ concentrated ambient PM. Because other researchers have found few if any cellular or biochemical changes in the lavage fluid of subjects exposed to concentrated ambient PM, we have dropped bronchoalveolar lavage from our study protocol. Therefore, only blood coagulability indices, electrocardiogram and heart rate/heart rate variability changes, and pulmonary function will be measured after each exposure. This simpler study aim will allow us to complete a new goal of monitoring the changes in 12 normal subjects after exposure to air or concentrated ambient PM. This will be performed with comprehensive chemical characterization of the exposure atmospheres to permit us to more carefully use day-to-day variability in PM composition in the interpretation of biological results.

TOXICOLOGY STUDIES

Physicochemical Parameters of Combustion Generated Atmospheres as Determinants of PM Toxicity

PI: L.C. Chen (NYU)

Objective

This study examines the broad hypothesis that the toxicological effects associated with combustion-generated PM depend upon specific physicochemical characteristics of the particles. This project will determine the influence of physicochemical parameters, specifically those of combustion generated PM, on the time course, dose response, and persistence of particle-induced cardiopulmonary effects.

Progress

This project works closely with Dr. Nadziejko's PM Center-sponsored study in the measurement of cardiopulmonary effects upon exposures to various PM atmospheres.

We have developed two furnace systems to produce realistic combustion effluents. We have successfully produced a mixture of carbon, SO_2, and metal (iron or copper). This will allow determination of specific components, especially metals, which may be responsible for adverse health effects, and an assessment of whether any effects could be nonspecific, i.e., whether they will occur following the inhalation of any type of particle.

Both furnace systems and the electronics for temperature regulation were updated. To produce Fe (or Cu), and S-coated carbon particles, sucrose solutions containing varying concentrations of $Fe(NO_3)_3$ (or $Cu(NO_3)_2$) were generated and burned in a furnace system previously used to produce coal fly ash. The mass median diameter (MMD) of particles produced by a Collison nebulizer (before combustion) using 10 sucrose solutions (each containing 1117 ppm Fe) was 0.9 μm. When 10% sucrose solution containing 1117 ppm Fe (or Cu) was burned in the furnace at 750°C in the presence of 1 ppm SO_2, ultrafine particles with a median diameter of 32 ± 1.3 nm (34.0 ± 7.4 nm for Cu) and geometric standard deviation of 1.55 were produced. A number concentration up to 1.9×10^7 particles/cc was achieved. XRF was used to measure the concentrations of iron, copper, and sulfur in these particles. At this combustion condition, the particles produced from this furnace contained 35.1% and 3.6% by mass of iron and sulfur, respectively (30.6% copper and 6.9% sulfur when copper was used). It appeared that copper is almost twice as efficient (6.9% vs. 3.6%) in converting sulfur dioxide gas to particle-associated sulfur.

Sprague Dawley rats were exposed to furnace gas, or to 450 $\mu g/m^3$ of these particles in furnace gas, for 3 hours and their lungs were lavaged 24 hr post exposure. A lead oxide diffusion denuder was used to remove SO_2 from the exposure atmospheres. None of the

exposure atmospheres produced changes in LDH levels in the lavage fluid. However, those particles containing a mixture of iron, SO_2, and carbon produced a 6.8 fold increase over the furnace gas control in the total number of cells in the lavage, whereas particles containing copper, SO_2, and carbon did not produce any change in this parameter. The results are shown in the table below.

Exposure Atmosphere	**Total Cell Counts (10^6)**	**LDH (BB unit)**
Furnace Gas	0.70 ± 0.14	95.5 ± 10.2
SO_2 + carbon	1.52 ± 0.31	78.7 ± 6.3
Copper + SO_2 + carbon	1.52 ± 0.23	80.0 ± 13.5
Iron + SO_2 + carbon	4.77 ± 0.41*	113.7 ± 28.2

Values are mean ± SE, (n=4 to 7 per exposure group).
* Significantly different than furnace gas control ($p < 0.0001$).

Plans for Next Year

We will determine the irritant potency as well as the cardiopulmonary effects of these particles. Healthy animals will be used first, followed by compromised animals. The morphology of these particles will be investigated using the atomic force microscope, XRF, and transmission and scanning electron microscopes.

Effects of Particle-Associated Organic Irritants on the Cardiovascular System

PI: C. Nadziejko (NYU)

Objective

The effect of particulate matter (PM) on the cardiovascular system is an increasingly important public health issue. However, the physical and/or chemical properties of PM responsible for these serious health effects are currently unknown. The scope of this project has been expanded in response to recent epidemiological studies which suggest that co-pollutant gases contribute to the cardiovascular effects of PM. Therefore, experiments have been added to examine the cardiovascular effects of gaseous co-pollutants. This project is closely coordinated with that directed by Dr. Chen also as part of the PM Center. The same animal models and biological endpoints are used in both studies. However, the two projects differ in the exposure atmospheres that are being examined, so as to test different hypotheses regarding the biologically active components of PM.

Progress

Identification of sensitive animal models and endpoints: Although our primary objective is to identify the biologically active components of PM, sensitive animal models and endpoints are needed to achieve this goal. Human panel studies have reported an increase in arrhythmias in association with exposure to ambient PM. Therefore, there is a need for an animal model that not only has cardiac arrhythmias but that also has an increase in arrhythmia frequency with PM exposure. We implanted ECG transmitters in a group of male F-344 rats (n = 12) aged 18 months. Baseline studies showed that at least half of these rats had spontaneous arrhythmias consisting of ectopic beats and delayed beats. We then developed protocols for arrhythmia identification and quantification. As described below, there was a statistically significant increase in the frequency of one type of arrhythmia in these rats following exposure to concentrated ambient PM.

Effects of concentrated ambient PM, ultrafine carbon particles and SO_2 on cardiac parameters in young and old rats: While concentrated ambient PM is the most realistic surrogate for particulate air pollution for use in controlled animal and human exposures, it is not enriched in ultrafine particles and it does not contain co-pollutant gases. We have done a series of experiments comparing the effects of concentrated ambient PM, laboratory-generated ultrafine carbon particles, and SO_2 in normal and aged rats. These studies were done to determine whether the effects of any of these exposures are similar to cardiac health effects reported in panel and epidemiology studies of particulate air pollution. We have exposed young and aged rats to concentrated ambient PM for 4 hrs with EKG monitoring before, during, and up to 48 hrs exposure. Effects of PM exposure on heart rate are shown below:

Rat age	**PM conc. (µg/m³)**	**Effect on heart rate**	**Time course of effect**
Young	215	Decrease	6-23 hrs post exposure
Young	60	Increase	1-24 hrs post exposure
Old	200	Increase	18-25 hrs post exposure
Old	161	Decrease	22-31 hrs post exposure

Significant effects on heart rate were seen in each exposure but the direction of the effect was variable. Heart rate effects occurred within the first 24 hours after exposure in young animals and lasted for 18-24 hrs. Heart rate effects in aged rats were delayed in onset and shorter in duration. Exposure to ultrafine carbon (generation and characterization as described in Project 3) at 1500 and 500 $\mu g/m^3$ significantly decreased heart rate in young rats and increased heart rate in old rats with a similar time course of effects as for PM exposure. Exposure to SO_2 at a concentration of 1 ppm had no significant effect on heart rate, even though exposures were repeated twice in young rats and four times in old rats (using a crossover design). Analysis of arrhythmias in aged rats showed that exposure to concentrated ambient PM, ultrafine carbon or SO_2 had no significant effect on the frequency of ectopic beats. However, PM exposure significantly increased the frequency of sinus node arrythmias

(skipped beats, prolonged pauses and irregularly-irregular sinus rhythm). Surprisingly, an episode of prolonged EKG pause was also seen in two young rats after PM exposure, despite the fact that this arrhythmia is rare even in old rats.

Conclusion: Heart rate changes were seen following inhalation of particles (concentrated ambient PM, ultrafine carbon and tobacco smoke), but not in response to the co-pollutant gases CO, NO_2 or SO_2. However, only concentrated ambient PM (and not ultrafine carbon) was found to increase the frequency of arrhythmias. Spontaneously hypertensive rats and aged rats appear to be sensitive to inhaled ambient PM in terms of changes in heart rate and arrhythmia frequency, respectively.

Plans for Next Year

A new group of spontaneously hypertensive rats has been implanted with blood pressure transmitters. We are developing protocols for arrhythmia analysis from blood pressure tracings to examine arrhythmia frequency in hypertensive rats. Aged F-344 rats are not currently available, but a group of 18 month old Sprague Dawley rats as well as a control group of young rats has been implanted with EKG transmitters. These three groups are currently being used in studies of fine and ultrafine sulfuric acid aerosols. Additional studies will be done with carbon particles and acid-coated carbon particles. This series of experiments will provide information on the relative roles of particle acidity, particle size, and physical structure in cardiac effects of PM.

The Role of PM-Asssociated Transition Metals in Exacerbating Infectious Pneumonia

PI: J.T. Zelikoff (NYU)

Objective

The objective of this study is to determine the role that PM-associated transition metal solubility and concentration might play in bringing about previously-observed changes in host immunocompetence. To this end, *Streptococcus pneumoniae*-infected rats are exposed to artificially-generated atmospheres containing soluble/insoluble forms of individual metals found at the highest concentrations in immunoreactive New York City (NYC) PM samples. Similar studies will then employ mixtures of the soluble/insoluble forms of the metals so as to ascertain whether the immunosuppression in PM-exposed hosts is dependent, at least in part, upon any interactions between the metals present. Immunotoxic effects of metal exposure on non-infected animals are also examined in these studies so as to determine the extent to which the PM-associated metals are capable of producing immunodysfunction in and of themselves.

Progress

So as to determine relevant metal concentrations to use in these studies, NYC air particulates collected upon filters were extracted and the concentration (ppb) of five metals that have been shown in previous air pollution studies to be associated with PM were determined using graphite furnace atomic absorption spectroscopy. Zinc (Zn), manganese (Mn), copper (Cu), iron (Fe), and nickel (Ni) were present in the following order of concentration (total ng / sample weight): Cu > Zn > Fe > Ni > Mn.

Studies employing uninfected 8-mo-old F-344 rats examined the immunological, histological, and biochemical changes associated with a single 5 hr inhalation exposure to $FeCl_2$ at a concentration of ~100 $\mu g / m^3$; effects were examined 1, 18, and 48 hr post-metal exposure. In the absence of effects upon lavaged cell profile, viability, cell number, or lavageable LDH / protein levels, animals examined 1 hr post-exposure demonstrated altered blood cell profiles. Percentages of lymphocytes and monocytes in Fe-exposed animals were 2- and 3-fold lower, respectively, while neutrophils were 3-fold higher as compared to controls; by 18 hr post-exposure, differential blood counts returned to control levels. This time-related response is identical to that seen in our earlier NYC PM studies and may indicate a particle-induced stress response. At the same 18 hr post-exposure timepoint, proliferation of splenic T-lymphocytes was decreased in Fe-exposed animals (compared to control), revealing effects upon the systemic immune response. At all post-exposure timepoints, superoxide anion ($O_2^{\cdot -}$) production by macrophages (Mø) recovered from Fe-exposed rats was enhanced compared to production from control cells. Effects upon hydrogen peroxide (H_2O_2) production by Mø were dependent upon time post-exposure.

In another series of studies, rats were infected with *S. pneumoniae* 48 hr prior to exposure to either air or Fe. Rats were sacrificed either just before initiation of exposure or 18 hr later and effects upon circulating blood cell profile and pulmonary bacterial burdens determined. Eighteen hr following exposure, percentages of blood neutrophils were similar in uninfected animals and in infected, air-exposed control rats. However, infected animals exposed for 5 hr to Fe and examined 18 hr later had a 37% increase in blood neutrophils and a 33% drop in lymphocytes (compared to air controls). Moreover, while virtually all bacteria were cleared from the lungs of infected air-exposed rats by 48 hr post-infection, bacterial burdens in Fe-exposed animals were increased 10,000-fold (compared to those rats scarified immediately prior to exposure). Since *S. pneumoniae* are iron-sequestering organisms, it is difficult to determine whether Fe-induced changes in burdens were due to effects upon the bacteria itself, to alterations in immune mechanisms responsible for the clearance of the pathogen, or a combination of both factors. In another experiment in which already-infected rats were examined 3 and 18 hr following Fe exposure, percentages of blood neutrophils were significantly reduced (compared to control) 3 hr following exposure, but returned to control values by 18 hr. In this same study, while no difference in lavaged cell profile was observed 3 hr post-exposure, rats exposed to Fe had significantly greater numbers of lavaged Mø and fewer lymphocytes and neutrophils than control animals by 18 hr.

Uninfected or *S. pneumoniae*-infected rats were exposed for 5 hr to $MnCl_2$ at a concentration of ~100 $\mu g/m^3$. In all cases, any observed effects from Mn upon lymphoproliferation, blood/lavage cell profiles, and oxyradical production were only seen 1 hr post-exposure. For example, splenic T-cell proliferation and PMA-stimulated $O_2^{\cdot -}$ production were both modestly elevated at this single timepoint. Moreover, as observed following Fe exposure, the percentages of circulating neutrophils and monocytes increased, while lymphocyte levels dropped compared to control.

S. pneumoniae-infected rats exposed to $MnCl_2$ for 5 hr and sacrificed 18 hr post-exposure had a greater increase in bacterial killing/clearance (as compared to control. Because of the antibacterial properties of some forms of Mn, it is unknown whether changes in bacterial burdens were due to direct effects upon the pathogen, Mn-induced alterations in antimicrobial immune defense mechanisms, or a combination of both factors.

Plans for Next Year

Studies examining the effects of metal combinations on host antimicrobial defense mechanisms will be performed. Studies are currently underway to determine the effects of Fe and Mn mixtures on the same immunological endpoints examined for each metal alone. In addition, studies will assess the effects of insoluble salts of these same five metals (i.e., Fe, Mn, Ni, Zn, and Cu), alone and in combination, so as to determine whether it is the soluble or insoluble metal forms that play the most important role in particle-induced effects upon antimicrobial lung defenses.

Immunomodulation by PM: Role of Metal Composition and Pulmonary Phagocyte Iron Status

PI: M.D. Cohen (NYU)

Objective

Particulate matter (PM) has been shown in epidemiological analyses to induce/exacerbate infectious lung disease, e.g.., pneumonia, and in toxicological studies to alter the manner by which the lung handles bacterial infections. Metals, a major constituent of PM, are a class of toxicants known to be immunomodulatory. Several metals produce effects upon directly-exposed immune cells (i.e., pulmonary macrophages [PAM] and neutrophils [PMN]) that result in a decrease in their antibacterial activities in the lungs. However, it remains unclear which of the many metals present in PM (alone or in combination) might be responsible for any effects on leukocyte function, or the mechanism(s) of action under-lying these effects.

Among the many metals commonly present in PM is iron (Fe), an important micronutrient for both the growth of bacteria *in situ* as well as for maintenance of PAM and

PMN antibacterial function. This new study proposes that: (1) PM modulates the antibacterial function of lung phagocytes by altering their intracellular Fe status, in part, by affecting homeostatic mechanisms that assure tight control of cellular Fe levels so as to permit optimal antibacterial activity; and (2) the relative (with respect to other metals present) content of Fe in PM governs whether this alteration involves either a direct effect or an indirect subversion of these mechanisms. For example, in PM with a high relative content of Fe, uptake/slow dissolution of insoluble Fe from PM in conjunction with increased cellular deposition of soluble Fe (due to normal lacto- [LF] or transferrin [T] activity) will lead to Fe overload in leukocytes and decreased antibacterial function. Conversely, in PM containing a low/very low Fe content, the presence of relatively greater amounts (with respect to Fe itself) of three major potential competitors for intrapulmonary T-/LF-binding (e.g., aluminum [Al], manganese [Mn], and vanadium [V]) will bring about a state of intracellular Fe deficit and reduced antibacterial function as a result of inhibited transport of endogenous Fe to PAM/PMN.

Progress/Plans

In this new study, $PM_{2.5}$ with varying metal contents will be obtained with cooperation from PM Centers in Los Angeles and Seattle; $PM_{2.5}$ particles will be collected onto filters held in dichotomous samplers. The resulting filters from each site will be subdivided and pooled for use in two major objectives to test the aforementioned hypothesis: (1) To determine the Fe status of PAM and PMN isolated from lungs of rats instilled with regional PM. $PM_{2.5}$ present on pooled portions of all filters from a given site; and (2) To assess whether regional PM-related changes in PAM/PMN intracellular Fe status can give rise to modifications in their antibacterial function.

All regional PM samples will be characterized for respective Fe, Al, Mn, and V content. This information will be critical in validating the role that content relationship among these T-/LF-binding metals in the samples may have in the mechanism(s) underlying effects on leukocyte Fe status and anti-bacterial function. Furthermore, this data will permit future studies, wherein artificially-generated $PM_{2.5}$ containing only Al, Fe, Mn, and V at the levels present in each sample are instilled into rats and the timeframe and magnitude of any effect upon PAM/PMN Fe status/antibacterial function assessed, to verify that it is the metals in each regional sample (rather than organics, endotoxin, etc.) that are responsible for any observed effects.

CENTER SERVICE CORE

Coordinator: L.C. Chen (NYU)

XRF Instrument Validation and Optimization

With the recruitment of Dr. Polina Maciejczyk, a post-doctoral fellow, we have made considerable progress in setting up the XRF measurement system. Several modifications were made: one secondary fluorescers turret wheel was re-aligned, which significantly increased the analytical sensitivity. Extensive work was done to decrease noise and to improve resolution. Currently, the instrument was calibrated for quantitation of 25 elements. In addition, the performance of the manufacture's suggested procedure for determining Sb, Cd and Sn was deemed to be unacceptable. Thus, a new secondary Cs flourescer was constructed and a new procedure was developed. Although most likely these elements will not be detected in ambient air, noticeable improvement was achieved in lowering the detection limits for the aforementioned elements. The XRF instrument is currently at the stage of validation.

Samples obtained from other XRF facilities (RTP, Chester Labs, NIST, and Oregon Dept. of Environ. Quality) are being quantified as a part of an intercomparisons study. The agreement is quite good for the first row transition elements (<10%). Lighter elements show a lesser agreement (30%), and He flush failure is a primary suspect. 5th and 6th row elements are hardly present in ambient air samples, and report very high uncertainties (50-100%). Samples with loadings of latter elements will be arriving from Chester Labs within next few weeks, and will facilitate the validation procedure.

We will explore alternative methods to analyze the "difficult" elements of interest (Se, Sb, Cd) using their L-lines instead of the K-alpha lines to obtain the desired (lower) detection limits. Furthermore, contaminant peaks from target wheel (Ag) and collimator (Sn) will be remedied by covering everything with W or Zr foil.

We will participate in the inter-comparison study with other established XRF facilities to further validate the instrument and establish the PM Center as a reliable XRF analysis lab.

Ion Chromatography

Ion Chromatography (IC) has been operational since June, 1999. During this period, routine IC analysis to measure concentrations of nitrate and sulfate were performed for Center Investigators. For Dr. Thurston, sulfate concentrations from approximately 1000 air filter samples taken from 9 cities in the US were measured. For Dr. Chen, sulfate and nitrate concentrations of over 200 samples collected from a combustion system were measured. It is anticipated that this instrument will continue to be used as a routine measurement system for soluble ions.

Mobile Air Monitoring Van

A study, which is partially funded by the PM Center, has been initiated which involves measuring air quality at various sites in the South Bronx of NYC for comparison to measures obtained at a central monitoring site. For this purpose, a mobile monitoring van will be used. The van was overhauled mechanically with new tires, brakes, exhaust manifold, and A/C systems. Overall performance and safety checks were also performed. For the current "Air Quality in the South Bronx" study, equipment is currently being installed in the van to monitor concentrations of PM_{10}, $PM_{2.5}$, black/organic carbon, ozone, nitrogen oxides, sulfur dioxide, and carbon monoxide. This equipment is as follows:

Tapered Element Oscillating Microbalance: Direct, continuous measurement of particulate mass will be accomplished using TEOM technology. The TEOM has received US EPA certification for continuous PM_{10} monitoring and is also capable of measuring $PM_{2.5}$. Concentration data are reported in $\mu g/m^3$ at standard averaging times of 10 min, 30 min, 1 hr, 8 hr, and 24 hr. The instrument includes software to view results and control system operation from an on-board personal computer via a serial port connector, providing real-time outputs for quality control checks. We also purchased an Automatic Cartridge Collector to collect ambient PM samples for elemental analysis using XRF.

Aethalometer, RTA9-E1-Man-b, Andersen Instrument Corporation: Black ("elemental") carbon, a key component of diesel exhaust, will be measured by the AE-14U aethalometer (Magee Scientific, Boulder Co). The instrument detection limit is 1 ng of elemental carbon at temporal resolution selectable from one minute to one hour. It includes an embedded computer so that the data can be downloaded directly to an onboard PC in the van.

Carbon Monoxide Monitor, Model 48C, Thermo Environmental Instrument Inc.

Multigas Calibrator, Model 146C-432-111, with permeation oven and zero air generator, Thermo Environmental Instrument Inc.

Monitor Weather Station II, Product #73545.001, Davis Instruments

Nitrogen oxides Monitor, Model 8840, Monitor Labs.

Sulfur Dioxide Monitor, Model 8850, Monitor Labs.

Ozone Monitor, Model 103-PC, Thermo Environmental Instrument Inc.

Size-Selective Sequential Daily Air Sampler

The data collected from these instruments will be logged into a computer equipped with the "Lab View" data acquisition system, which will expedite data handling and transfer. Data will also be downloaded to a lap top computer once every week and backed up to a central server.

PUBLICATIONS AND PRESENTATIONS OF THE PM CENTER

PUBLICATIONS

Gwynn, R.C. and Thurston, G.D. 2001. The Burden of Air Pollution: Impacts in Racial Minorities. Environ. Health Perspect. (In press).

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Zelikoff, J.T., Chen, L.C., Cohen, M.D., and Schlesinger, R.B. 2001. The toxicology of inhaled woodsmoke. J. Toxicol. Environ. Health (In press).

ABSTRACTS/PRESENTATIONS

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Chen, L.C., Su, W.C., Qu, Q., Cheng, T.J., Chan, C.C., and Hwang, J.S. 1999. Composition of particulate matter as the determinant of cellular response. Presented at 3rd Colloquium on Particulate Matter and Human Health, Durham, NC, June, 1999.

Chen, L.C., Su, W.C., Wesselkamper, S., Nadas, A., Hu, W., Tang. E., and Gordon, T. 2001. Genome-wide expression monitoring in rats exposed to particles and ozone. Am. J. Respir. Crit. Care Med. 163:A173. Presented at American Thoracic Society International Convention, San Francisco, CA, May, 2001.

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Nadziejko, C. Quantitative analysis of arrhythmias. 2001. Presented at the Workshop on Cardiovascular Effects Associated with Air Pollution, Rochester, NY, March, 2001.

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Wesselkamper, S., Chen, L.C., and Gordon, T. 2000. Genetic modeling of tolerance to zinc oxide inhalation in inbred mice. Toxicol. Sci. 54:320. Presented at Society of Toxicology Annual Meeting, Philadelphia, PA, March, 2000.

Wesselkamper, S., Chen, L.C., Kleeberger, S.R., and Gordon, T. 2000. Genetic variability in the development of pulmonary tolerance to zinc oxide in inbred mice. Presented at American Thoracic Society International Convention, Toronto, Ontario, Canada, May, 2000.

Won, C., Cook-Granroth, J., Hoffman, E.A., and Cohen, B.S. 2000. X-ray CT-based assessment of variations in human airway geometry and the implications for the evaluation of particle deposition and dose to different populations. Presented at Scientific Assembly and Annual Meeting of the Radiological Society of North America, Chicago, IL, November, 2000.